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Acetylsalicylic acid therapy: Influence of metformin use and other variables on urinary 11-dehydrothromboxane B_2 levels

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ABSTRACT

Background: The effect of acetylsalicylic acid (ASA) may be measured through the analysis of urinary concentrations of 11-dehydrothromboxane B_2 (11-dhTXB_2), a metabolite of thromboxane A_2, which is a potent platelet aggregant agent. It has been suggested that metformin (an oral antidiabetic drug) could improve oxidative stress and control platelet activation in type 2 diabetic patients, potentially reducing cardiovascular risk. We determined the concentrations of urinary 11-dhTXB_2 in type 2 diabetic patients taking ASA and its concentrations with metformin use and several other clinical variables (hypertension, age, gender, smoking, body mass index, insulin and statin use), considering a reduction of at least 75% in the concentrations of this marker as a target, compared to results before ASA intake.
Methods: Urinary concentrations of 11-dhTXB_2 of 81 type 2 diabetic patients were measured before and at 15 days taking 100 mg of aspirin daily.
Results: Most patients who presented a reduction of 11-dhTXB_2 above 75% were under metformin use. This reduction was achieved in 51.5% of patients taking this drug, against 20.0% in the patients who were not ($p = 0.027$). The analysis of the other variables did not show a significant difference. The use of metformin appears to play a role in the reduction of 11-dhTXB_2 concentrations in type 2 diabetic patients.
Conclusion: According to previous reports, hyperglycemia control seems to be a determinant factor for the success of ASA therapy, given the influence of metformin in the reduction of 11-dhTXB_2 concentrations.

1. Introduction

Diabetes mellitus type 2 is a metabolic disorder largely associated to microvascular and macrovascular complications and an enhanced risk for cardiovascular disease (CVD). Several clinical variables, including hypertension, hyperglycemia, dyslipidemia, C-reactive protein and body mass index, increase the risk of late complications [1]. According to International Diabetes Federation, cardiovascular disease is the major cause of mortality and morbidity in type 2 diabetic patients, which is confirmed by several epidemiological reports [2].

Acetylsalicylic acid (ASA), a nonsteroidal anti-inflammatory drug, is the most commonly antiplatelet agent used due to its low cost and relative lack of adverse effects, when administered in low doses [3]. It impairs the platelet formation of thromboxane A_2, a potent vasoconstrictor and platelet aggregant agent, through the acetylation of cyclooxygenase 1 (COX-1) in the serine-530 position, thus preventing the aracdonic acid binding to the enzymatic active site. Since this inhibition is irreversible, and platelets have no DNA, they are no longer able to produce new COX enzymes [4–6].

Metformin, an oral antidiabetic drug, is widely used by type 2 diabetic patients as an auxiliary therapy for control of hyperglycemia. It is classified as a biguanide, and its mechanism of action is related to both a decrease in hepatic glucose production and intestinal absorption, as an enhancement in insulin sensitivity due to increase in peripheral glucose uptake and utilization [7].

2. Methods

2.1. Patients

Eighty-one type 2 diabetic patients were enrolled at this study, according to the following inclusion criteria: (1) diagnosis of diabetes mellitus type 2, made under the American Diabetes Association (ADA, 2006) criteria: ≥2 fasting glucose ≥126 mg/dl; random glucose ≥ 200 mg/dl; oral glucose tolerance curve ≥ 200 mg/dl at

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120 min; (2) not in use of ASA before first urine collection;(3) start the intake of ASA immediately after the first urine collection. The exclusion criteria were: use of nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs, antiplatelet agents, oral anticoagulant agents, heparin and antiacids; chronic kidney disease, hepatic insufficiency, acute infectious disease, inflammatory, autoimmune or myeloproliferative disease; surgery in the last 6 months or pregnancy.

After the first urine collection, all patients started therapy consisting in taking 100 mg of ASA daily. After 15 days, another urine sample was collected. The urine samples were obtained from the first morning urine, or after 4 h of urinary retention. The samples were centrifuged at 1500 rpm for 10 min and the supernatants were aliquoted and stored at −80 °C until the analysis moment.

2.2. Clinical data

Data referring to drug intake such as oral antidiabetic drugs, insulin and statin, besides the epidemiologic and clinical variables, as gender, age, smoking habit, BMI and hypertension (individuals in use of antihypertensive drugs were classified as hypertensive [8]) were obtained from clinical records.

2.3. Analysis

The 11-dhTXB$_2$ quantification was made by an immunoenzymatic assay (ELISA), using the 11-dehydrothromboxane B$_2$ EIA kit (Cayman Chemical Co.) following the fabricant instructions. This assay is based on the competition between the 11-dhTXB$_2$ and the conjugate 11-dehydroTXB$_2$-acethilcholinesterase (ACE) for a limited number of binding sites of rabbit anti-serum specific for 11-dhTXB$_2$. The concentration of the conjugate is constant, and its binding to the sites is inversely proportional to the concentration of 11-dhTXB$_2$ in the sample.

The quantification of urinary creatinine (for standardization of the results, expressed as pg of 11-dhTXB$_2$/mg of creatinine) was carried out by a kinetic-colorimetric method, according to kit's manufacturer instructions. The statistical analysis was performed by using Pearson χ^2 test, and a <0.05 was significant between groups.

3. Results

Among the 81 patients enrolled at the study, 66 (81.5%) were under metformin use, and 42 (52%) were using both metformin and insulin. In the group of patients under metformin use, a reduction of at least 75% in concentrations of 11-dhTXB$_2$ was found in 51.5% of them, against 20% in the patients who were not in use of this oral antidiabetic drug ($p = 0.027$; OR = 4.25). For other drugs such as insulin and statin as well as epidemiologic/clinical variables, including gender, age, smoking habit, hypertension and BMI no difference was observed as groups were compared (Tables 1–3).

Table 2
Reduction of urinary 11-dhTXB$_2$ levels in type 2 diabetic patients under metformin intake.

	Reduction in urinary 11-dhTXB$_2$ levels		
	≥75%	<75%	Total
Metformin intake	34 (51.5%)	32 (48.8%)	66
No metformin intake	3 (20%)	12 (80%)	15
Total	37 (45.7%)	44 (54.3%)	81

$p = 0.027$, by Pearson χ^2; OR = 4.25.

4. Discussion

Based on the results, among all variables evaluated in this study, the use of metformin, an oral antidiabetic drug, seems to be that of greatest influence in the reduction of urinary 11-dhTXB$_2$ concentrations in type 2 diabetic patients under ASA therapy.

The associated therapy with these 2 drugs appears to enhance the effect of ASA due to a reduced platelet activity induced by metformin use. Metformin intake is frequent by type 2 diabetic patients as an auxiliary therapy for improving glycemic control in addition to diet and insulin use. According to previous reports, metformin by itself shows an antiaggregant activity, which can explain the reduction of 11-dhTXB$_2$ excretion [7]. The mechanism of action of ASA, by which it irreversibly impairs the TXA$_2$ formation is well known, but there are still controversies about the mechanism by which metformin reduces platelet aggregation. It is likely that this occurs through reduction in oxidative stress, but the antioxidant activity of metformin is not fully understood. It is suggested that hyperglycemia enhances the generation of reactive oxygen species, which can lead to generation of several active prostanoids that stimulates platelet activation [7]. These facts strongly suggest that an indirect pharmacological synergism may occur between these drugs, which may be explored in the development of new therapeutic schemes for type 2 diabetes treatment and in prevention of type 2 diabetes complications.

Although the other variables observed in this study do not provide conclusive data about their contribution in the achievement of the urinary 11-dhTXB$_2$ reduction goal, they are very important for the prognostic of type 2 diabetes and its associated complications and should always be considered in patients' management.

Table 1
Urinary 11-dhTXB$_2$ levels before and at 15 days of ASA therapy according to gender, hypertension, smoking habit and BMI.

Patient characteristics	Median (IQR) 11-dhTXB$_2$ before aspirin intake (pg/mg creatinine)	Median (IQR) 11-dhTXB$_2$ after aspirin intake (pg/mg creatinine)	p*	Reduction (%)	p
Men (n = 23)	151.00 (104.00–205.00)	52.00 (33.00–70.00)	0.00	66	0.026**
Women (n = 58)	204.00 (130.75–319.75)	51.00 (32.00–80.25)	0.00	75	
p**	0.107	0.814			
Hypertensive (n = 69)	179.00 (117.50–284.50)	52.00 (33.50–81.00)	0.00	71	NS**
Nonhypertensive (n = 12)	179.50 (60.75–314.75)	46.50 (22.75–76.75)	0.015	74	
p**	0.852	0.563			
Smokers (n = 9)	268.00(215.00–477.00)	68.00 (34.00–161.50)	0.021	75	NS**
Ex-smokers (n = 18)	179.50 (97.50–276.75)	64.00 (42.75–88.25)	0.001	64	
Nonsmokers (n = 54)	164.00 (117.00–283.75)	45.50 (31.75–72.00)	0.00	72	
p***	0.165	0.201			
BMI ≤ 25 kg/m^2 (n = 18)	163.00 (82.50–210.75)	63.00 (31.75–99.00)	0.005	61	0.028***
BMI 26–30 kg/m^2 (n = 21)	174.00 (101.00–335.00)	51.00 (34.50–74.00)	0.00	71	
BMI > 30 kg/m^2 (n = 42)	208.00 (147.50–147.50)	48.00 (30.50–78.75)	0.00	77	
p***	NS	NS			

IQR = Interquartile range.
* Wilcoxon test.
** Mann–Whitney test.
*** Kruskal–Wallis test.

Table 3
Association of the reduction in urinary 11-dhTXB$_2$ levels with gender, age, BMI, hypertension, insulin, statin use and smoking habit.

	Reduction in 11-dhTXB$_2$ urinary levels		
	≥75%	<75%	Total
Gender			
Women	30 (51.7%)	28 (48.3%)	58
Men	7 (30.4%)	16 (69.6%)	23
p = NS by Pearson χ^2 test			
Age			
<50 y	5 (29.4%)	12 (70.6%)	17
≥50 y	32 (50%)	32 (50%)	64
p = NS by Pearson χ^2 test			
BMI			
<25 kg/m^2	6 (30%)	14 (70%)	20
25–30 kg/m^2	11 (44%)	14 (56%)	25
>30 kg/m^2	20 (55.6%)	16 (44.4%)	36
p = NS by Pearson χ^2 test			
Hypertension			
No	6 (50%)	6 (50%)	12
Yes	31 (44.9%)	38 (55.1%)	69
p = NS by Pearson χ^2 test			
Statin			
Yes	22 (48.9%)	23 (51.1%)	17
No	15 (41.7%)	21 (58.3%)	64
p = NS by Pearson χ^2 test			
Insulin			
Yes	21 (42%)	29 (58%)	50
No	16 (51.6%)	15 (48.4%)	31
p = NS by Pearson χ^2 test			
Smoking			
Non-smoker	25 (46.3%)	29 (53.7%)	54
Ex-smoker	7 (38.9%)	11 (61.1%)	18
Smoker	5 (55.6%)	4 (44.4%)	9
p = NS by Pearson χ^2 test			
Total	37 (45.7%)	44 (54.3%)	81

It has been reported that elevated blood pressure can activate platelets, resulting in less efficacy of a standard dose of ASA [9,10]. Likewise, age contributes to greater platelet activation [11]. Similarly, platelet activation was higher in smoker and obese patients [12,13]. Concerning the gender, it is related that women respond less to ASA therapy than men [14], although this study was unable to confirm this result. Regarding insulin and statin use, a reduction in platelet activation in patients under use of these drugs has been reported by literature [15,16]. In the present study, this reduction does not reach a significant concentration, although a slight tendency was observed. The absence of significant difference for the variables age, gender, BMI, insulin and statin use may be attributed to the sample size, that is considered as a limitation of the study. However, the present study was able to identify the potential benefit of an oral antidiabetic drug use on the reduction of platelet activation, which can be translated by a reduction of ≥75% in urinary 11-dhTXB$_2$ concentrations. A greater reduction in urinary 11-dhTXB$_2$ concentrations was observed in patients whose baseline concentrations of this biomarker were higher, such as women, smokers and individuals with BMI >30 kg/m^2 compared to men, non-smoker and patients with BMI <25 kg/m^2, respectively.

Based on these findings, it is possible to suggest that a good glycemic control may be decisive for decreasing platelet activation and enhancing ASA efficacy, which may contribute to prevent atherothrombotic events.

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